CORNELL FELINE HEALTH CENTER NEWS

No. 5 **November 1981**

Semi-automated, kinetics-based ELISA for detection of feline coronaviral antibodies

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A major research objective of the Cornell Feline Health Center is a further understanding of the relationship between viremia and the immune response in feline infectious peritonitis (FIP), leading to the development of a serodiagnostic test for distinguishing cats with FIP from coronaviral antibody-positive cats with other diseases. As all veterinarians are aware, diagnosis of FIP and interpretation of coronaviral antibody titers is an area fraught with misunderstanding and controversy, so that development of such a test will be especially helpful in dispelling much of the mystery which currently surrounds coronaviral infections in cats.

In the course of this research a kinetics-based enzyme-linked immunosorbent assay (ELISA) for feline coronaviral antibodies using Gilford EIA System 50 technology has been developed in our laboratory. Following extensive testing this assay has shown good correlation with the immunofluorescent procedure currently in use at the New York State Diagnostic Laboratory at Cornell University. Several modifications of standard ELISA protocols were found necessary for coronaviral antibody specificity and elimination of false-positive reactions. These modifications and a complete description of the assay are the subject of a paper in preparation.[1]

In brief, the ELISA utilizes transmissible gastroenteritis virus (TGEV) of swine, a coronavirus previously shown to be cross-reactive with FIP virus, as target antigen. Polystyrene-copolymer cuvette strips are coated with a clarified preparation of diluted tissue culture medium and dried in an unhumidified incubator. Coated strips can then be stored at 4°C until needed.

Following addition of diluted test serum to the cuvettes and appropriate incubation conditions, the samples are allowed to react with a peroxidase-linked antibody conjugate which detects feline antibodies that have complexed with TGEV. A solution containing hydrogen peroxide and a color indicator (ABTS) is then added, and the development of color within the wells is followed at a wavelength of 405 nanometers for 15 minutes. This color reaction occurs when the substrate (hydrogen peroxide) is reduced and the color indicator is subsequently oxidized, due to the action of bound peroxidase.

The substrate reaction proceeds in a linear fashion with time, so that the slope of the reaction (i.e., the reaction rate) is directly proportional to the amount of coronaviral antibody present (i.e., the titer). Thus the steeper the slope of the substrate reaction, the higher the coronaviral antibody titer of the serum.

Absorbance readings are recorded every five minutes during the reaction and a printout is provided. Thus with this semi-automated ELISA an objective evaluation of

(Cont. on page 8)

Management of diabetes mellitus in the cat

N. Sydney Moise, D.V.M.

Diabetes mellitus is a recognized disease entity in middle-aged and older cats. Both male and female cats are affected. A diabetic cat is diagnosed by clinical signs, physical examination, and persistent hyperglycemia and glucosuria. Diagnosis is not based on a single elevated blood glucose because stressed cats can have temporary glucose levels as high as 225 mg/dl.[1]

Historical signs of 10 diabetic cats examined during the past year at the New York State College of Veterinary Medicine (NYSCVM) included polydipsia, polyuria, lethargy, polyphagia, weight loss, anorexia, rear limb weakness, diarrhea, and vomiting. Signs compatible with chronic infection, such as chronic cystitis and chronic glossitis and gingivitis, were also seen. Three of the cats had received excessive doses of megestrol acetate (Ovaban®) just prior to the onset of clinical signs. This uncommon drug complication has been sporadically described in cats.[2,3,4]

Physical examination revealed most cats to be obese; however, cachetic cats were seen when the owners were tardy in seeking veterinary attention. Hepatomegaly was commonly found, and icterus was found in one cat. Other physical examination findings included renomegaly, splenomegaly, feline endocrine alopecia, and muscle wasting.

Differential diagnosis in the cat with the above historical and physical examination findings includes diabetes mellitus, hyperthyroidism, hyperadrenocorticism, hepatic disease, renal disease, pancreatitis, feline infectious peritonitis, and neoplasia.

Results of laboratory data typical of the diabetic cats included hyperglycemia, elevated liver enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase), hypercholesterolemia, mild azotemia, and decreased electrolytes (Na, K, Cl, P). Urinalysis results always revealed glucosuria and occasionally ketonuria. Fasting serum insulin levels were determined by radioimmunoassay in seven diabetic cats and 14 normal cats. Concomitant plasma glucose levels were also determined in these cats. Although the insulin levels in the diabetic cats were not different from the normal cats, the concomitant plasma glucose levels were elevated.

Recommendations for Treatment

After the diagnosis of diabetes mellitus has been made in a cat, careful consideration should be given to management. The following recommendations are based on preliminary investigations conducted at the NYSCVM:

1. The cat should be treated subcutaneously with 0.25 - 1.0 unit/kg of protamine zinc insulin once daily or 0.25 - 1.0 unit/kg of NPH insulin twice daily. Start with lowest dose.

Paired insulin and glucose levels were determined in five diabetic cats after NPH insulin was given subcutaneously. The highest serum insulin level occurred two to four hours post-injection and the lowest

CORNELL FELINE HEALTH CENTER NEWS
A publication of
Cornell Feline Health Center
NYS College of Veterinary Medicine
Cornell University, Ithaca, NY 14853

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The ultimate purpose of the Cornell Feline Health Center is to improve the health of cats everywhere, by developing methods to prevent or cure feline diseases, and by providing continuing education to veterinarians and cat owners. All contributions are tax-deductible.

Diabetes mellitus (Cont.)

plasma glucose level occurred four to six hours post-injection. The duration of action was 10 hours. Two diabetic cats given protamine zinc insulin subcutaneously had a peak action occurring 12 hours post-injection and the duration of action was 24 hours.

2. A diabetic cat can be accurately regulated if blood glucose values are determined at the peak of insulin action. We have found the Chemstrip-bG test strips (Bio-Dynamics, Inc., Boehringer-Mannheim Co., Indianapolis, Ind.) to be valuable for estimating blood glucose levels. The glucose values corresponded to actual plasma glucose determinations. With these strips only one drop of blood is necessary for glucose estimation.

3. Cats should be fed canned cat food at the time of insulin injection and six hours after NPH insulin or 12 hours after protamine zinc insulin.

4. Ideally, urine glucose should be checked just prior to the next dose of insulin. Increases or decreases in dosage can then be made accordingly. (See Table I.)

Many times it is difficult to obtain urine samples from cats. If a cat uses a litter pan, plastic wrapping paper (e.g., Saran Wrap®) can be placed over the litter. A drop of urine can then be salvaged for glucose determination. When urine glucose determination is not possible, the dose of insulin should be based on the previous day's dose and clinical signs.

5. Periodic rechecks are important, especially if diabetic regulation is a problem. Concurrent disease (chronic renal disease, hyperthyroidism) or decreases in exogenous insulin requirements may be identified. Some cats can be diabetic temporarily, and if insulin therapy is continued despite the decreased need, fatal hypoglycemia may result.

The diabetic cat can be managed. Knowing the time after injection when exogenous insulin peaks and its duration of action in each diabetic cat will prevent overdosing. **Overzealous attempts at tight control of hyperglycemia may also lead to an overdose of insulin.** Owner education is paramount to proper regulation.

[1]Schaer, M.: A clinical survey of thirty cats with diabetes mellitus. JAAHA 13:23-27, 1977.

[2]Hutchison, J.A.: Progestogen therapy for certain skin diseases of cats. Can Vet J 19:324, 1978.

[3]Pukay, B.P.: A hyperglycemia-glucosuria syndrome in cats following megestrol acetate therapy. Can Vet J 20:117, 1979.

[4]Werner, R.: Letter to the editor. Feline Pract 8:4, 1978.

The author thanks Dr. Tom Reimers for performing the insulin assays.

About the author: see page 8.

Table I. Adjustment of insulin dose based upon urine glucose levels.

Cat's Condition	Urine Glucose	Insulin Dose
Anorectic	0	Give no insulin.
Eating	0	Give no insulin.
Eating	0.1-0.25%	Repeat previous dose.
Eating	.5%	Repeat previous dose.
Polyphagic, Polydipsic, Polyuric	1%	Increase 1/2 unit.
Polyphagic, Polydipsic, Polyuric	2%	Increase 1 unit.

Micturition disorders in cats with sacrocaudal vertebral injuries

N. Sydney Moise, D.V.M. and James A. Flanders, D.V.M.

Injury to the sacrocaudal vertebrae and nerves is common in cats. The injury is usually encountered when the cat is hit by a car. Examination reveals a flaccid and analgesic tail. Further neurological findings are variable. Normal urination and defecation are frequently disturbed. The situation is distressing to owners and veterinarians, as frequently a cat is euthanatized because of the consequences of a "broken tail."

The medical records of the New York State College of Veterinary Medicine teaching hospital were examined from January 1980 to September 1981. During this period 20 cats were diagnosed radiographically with sacrocaudal vertebral dislocations (S_3-Cd_1), caudal vertebral fractures or dislocations (Cd_1-Cd_4), or sarcral vertebral fractures (S_2-S_3). Nine cats were male and 11 were female. Based on neurological examinations the cats were divided into four groups, each containing five cats. Although individual variations existed, common features were present within each group. There was no relationship between the neurological findings and the locations of the radiographic lesions.

Group I cats had flaccid and analgesic tails. Anal tone, perineal reflex, bulbourethral reflex, and pain sensation were normal. Micturition and defecation were normal. Prognosis was excellent.

Group II cats had flaccid and analgesic tails. Anal tone, perineal reflex, bulbourethral reflex, and pain sensation were normal. Disturbances of urination and defecation occurred for four to 15 days after the injury. During this recuperative period the cats would strain to urinate, but were unable to void urine. This action was interpreted as an awareness of bladder distention. Attempts to manually express urine from the cats' bladders were not successful. Steady pressure applied to the bladder surface could not overcome the urethral resistance.

After several days, small amounts of fecal material were passed. Urination started several days following the onset of defecation. Several of the cats were treated with aminopropazine fumarate (Jenatone® 10-15 mg BID orally). Based on clinical impressions, the medication did not cause improvement in the degree of urethral resistance. Aminopropazine fumarate is advocated for the relaxation of urethral sphincter tone. These same cats were also treated with bethanechol (Urecholine® 2.25-5 mg BID orally). Within two hours after receiving this medication the cats urinated. Bethanechol is a parasympathomimetic drug which has an affinity for receptors on the urinary bladder. This drug causes bladder contraction, if some parasympathetic innervation is intact. Prognosis with Group II cats was excellent.

Group III cats had flaccid and analgesic tails. Anal tone was poor, although the anus was usually not dilated and open. Pain perception around the anus and perineal area was absent. Perineal and bulbourethral reflexes were usually absent, although in some cats a weak response was seen. Micturition and defecation were abnormal for 20 to 60 days. At first the cats seemed unaware of bladder distention. The cats did not make attempts to urinate. The cats' bladders could not be manually expressed because of high urethral resistance. Cystometrograms performed on these cats were negative for a detrusor reflex (bladder wall contraction). The cystometrogram measures the changes in the intravesicular pressure which occur with detrusor contraction. Defecation occurred without the cats posturing to defecate. Feces hung from the rectum, unable to be broken off by the anus.

After several weeks, the cats began to posture to defecate and urinate. Small amounts of urine were passed while the cats

Micturition disorders (Cont.)

slept. Although feces were passed, urine was not voided. Subjectively, the anal tone seemed to improve in some of the cats. After several additional weeks, small amounts of urine were actively voided. None of the cats could totally empty the bladder. A residual urine volume of 25 to 40 ml was common. Most of the cats returned to a satisfactory state, although complete recovery was not usually made.

During the observation period of Group III cats, several drugs were tried in an attempt to correct the detrusor-urethral dyssynergia. Detrusor-urethral dyssynergia occurs if bladder contraction is absent, or inadequate, with an increased urethral pressure. Combinations of bethanechol and phenoxybenzamine (Dibenzyline® 1-2 mg TID orally), bethanechol and aminopropazine, and bethanechol and diazepam (Valium® BID orally) were given. Each combination was tried for four to 10 days. Also methocarbamol (Robaxin® 10 mg/kg TID orally) and dantrolene (Dantrium® 4 mg BID-TID orally) were tried. Although evaluations of the therapeutic value of these drugs were based solely on clinical impressions, the only drug which helped the cats' micturition problems was dantrolene. Dantrolene, a skeletal muscle relaxant, seemed to decrease the urethral resistance. The results were not remarkable; however, perhaps the correct dose was not given. Prognosis for return to a functional pet in Group III cats was fair.

Group IV cats had flaccid and analgesic tails. Anal tone was usually poor with the anus dilated. Some cats did not have such a profoundly flaccid anus. Pain perception around the anus and perineal area was absent. Perineal and bulbourethral reflexes were usually absent. Fecal and urinary incontinence were present. The bladders were easily expressed in these cats. Three of these five cats did not recover. One cat made a satisfactory recovery after 28 days, and one cat is still under observation after 48 days. Drug therapy was not of value in the cats treated. Prognosis for recovery in this group was poor.

Lesions of the sacral segments (gray and white matter), sacral nerve roots (dorsal and ventral), or peripheral sacral nerves (pelvic and pudendal nerves) alter the functions of the bladder and urethra. In the normal cat detrusor contraction synchronizes with urethral relaxation for micturition to occur. If the cell bodies in the sacral segments are destroyed, or if the nerves in the cauda equina are severed, the prognosis for recovery is grave. If minor injuries occur, recovery may take place. It is clinically difficult to know the type of injury sustained.

Sympathetic innervation from the lumbar segments also plays a role in normal micturition. A recent review article[1] describes the relationship between parasympathetic, sympathetic, and somatic innervations to the lower urinary tract. Various drugs have been advocated to treat different types of micturition disorders. Unfortunately, the response we have seen to date has been disappointing. Perhaps the correct drug dosages are not known for cats. In the limited number of cats we have observed, the animals' recoveries were most dependent on time and supportive care.

The management of a cat with a micturition disorder is difficult. If the bladder can be expressed, then this should be done three times daily. However, if the cat's bladder is difficult to express, the choice of management is either hospitalization with an indwelling urinary catheter or intermittent catheterization. We preferred the latter. Most of the cats adapted to this procedure without the need of tranquilization. Sterile technique should always be employed. The soft polyvinyl feline urinary catheters were preferred. The catheterizations may be continued until clinical improvement occurs or the cat is euthanatized. Antibiotics were used in all cats. Cystitis was not a major problem. Constipation was controlled with the following diet fed twice daily: 6 oz. canned cat food, one teaspoon Metamucil®, and three tablespoons Kellogg's Bran Buds®.

All of the cats' tails remained flaccid and analgesic. Several of the cats had tail amputations for ease in management. However, three cats had tail amputations

(Cont. on page 8)

Thank You

The Cornell Feline Health Center wishes to publicly extend our gratitude to the following veterinarians, who have participated in the memorial gifts program in 1981. We are deeply grateful for the confidence and support for our research that each memorial gift represents.

The funds from these contributions enable us to continue disseminating the latest discoveries in feline health care to practitioners and cat owners. In addition, this thoughtful gesture by the veterinarian following the death of a patient is an exceptional personal expression of sympathy to the client. It is a meaningful, constructive action at a time of extreme sensitivity, which imparts a hope for the future.

To all our friends listed here, our heartfelt thanks.

Bill Atkinson, D.V.M.; Denton, Texas

David H. Baldwin, V.M.D.; Vermont-New Hampshire Veterinary Clinic, Inc.; Brattleboro, Vt.

Samuel Bender, D.V.M.; Bronx, N.Y.

Dr. Richard N. Benjamin; Berkeley Dog & Cat Hospital; Berkeley, Calif.

Dr. Jay Berkowitz; Port Washington Animal Hospital, Inc.; Port Washington, N.Y.

Roy D. Bertolet, V.M.D.; Philadelphia, Pa.

Karen F. Bowls, D.V.M.; Atascadero, Calif.

Dr. R. Bradford; Mid-Peninsula Animal Hospital; Menlo Park, Calif.

Brockport Animal Hospital; Brockport, N.Y.

Burrstone Animal Hospital; Yorkville, N.Y.

Cat Clinic of Beaverton; Beaverton, Oreg.

Dr. Ching; Mid-Peninsula Animal Hospital; Menlo Park, Calif.

Milton P. Crenshaw, D.V.M.; Animal Medical Clinic of St. Paul; St. Paul, Minn.

Henry M. Dykehouse, D.V.M.; Argay Cat Clinic; Portland, Oreg.

Eastview Veterinary Clinic; Penn Yan, N.Y.

Dr. Jay W. Geasling; Sheridan Animal Hospital; Kenmore, N.Y.

Dr. William A. Gerber; Niagara Falls, N.Y.

Guilderland Animal Hospital; Altamont, N.Y.

Hasbrouck Heights Veterinary Clinic; Hasbrouck Heights, N.J.

Michael G. Henes, D.V.M., P.C.; Croton-on-Hudson, N.Y.

William H. Herbold, D.V.M.; W. Islip, N.Y.

Bernard S. Hershhorn, D.V.M.; New York, N.Y.

Dr. Linda C. Holste; Northgate Pet Clinic; Decatur, Ill.

Dr. Tim Howell; Cat Care Clinic; Indianapolis, Ind.

Dr. Gordon C. Johnson; Falls Village, Conn.

Dr. Paul Klinefelter; Waldo Animal Clinic, Waldo, Ohio

Dr. Barry Kopp; Greenwich Animal Hospital, P.C.; Greenwich, Conn.

Dr. & Mrs. Harold Kopp; Greenwich Animal Hospital, P.C.; Greenwich, Conn.

Malcolm A. Kram, D.V.M.; The Animal Clinic of New York; New York, N.Y.

Dr. Arthur Kronfeld; Port Washington Animal Hospital, Inc.; Port Washington, N.Y.

Allan A. Leventhal, D.V.M.; Bolton Veterinary Hospital, P.C.; Bolton, Conn.

Dr. Lawrence Leveson; Niagara Falls, N.Y.

Marcia J. Levine, D.V.M.; Summer Street Cat Clinic; Buffalo, N.Y.

Dr. John Loda; Feline Veterinary Hospital; Port Washington, N.Y.

Dr. R.E. Lynk; Delmar Animal Hospital; Delmar, N.Y.

Massapequa Hospital for Animals; Massapequa, N.Y.

Robert A. Mavian, D.V.M.; Millwood Animal Hospital Ltd.; Millwood, N.Y.

Dr. G. Memon; Meker Veterinary Clinic; S. Attleboro, Mass.

Dr. Eric W. Mendel; Valley Animal Hospital; Vestal, N.Y.

Dr. Jolene Nagakura; Topsfield Veterinary Hospital, Inc.; Topsfield, Mass.

Dr. James K. Olson; Alpine Veterinary Practice; Dillon, Colo.

Oradell Animal Hospital; Oradell, N.J.

Billie A. Pennings, D.V.M.; Cat Veterinary Clinic, P.C.; Houston, Texas

Dr. Earle Peterson; Raritan Animal Hospital; Edison, N.J.

Dr. Russ Petro; Valley Cottage Animal Hospital; Valley Cottage, N.Y.

Dr. Frank R. Powell; South Towne Veterinary Hospital; Rochester, N.Y.

Dr. Ralph L. Reese; Double R Veterinary Clinic; Calabasas, Calif.

Cynthia A.K. Rigoni, D.V.M.; Cat Veterinary Clinic, P.C.; Houston, Texas

Michael D. Rosen, D.V.M.; Feline Veterinary Clinic; Falls Church, Va.

Rutland Veterinary Clinic; Rutland, Vt.

Burton Saunders, D.V.M.; Spring Valley Animal Hospital; Monsey, N.Y.

Dr. Ned Schankman; Bethany Veterinary Hospital; Bethany, Conn.

Dr. Schulte; Mid-Peninsula Animal Hospital; Menlo Park, Calif.

M.V. Scott, D.V.M.; Potomac Animal Hospital; Potomac, Md.

Maurice E. Serling, D.V.M.; Larchmont, N.Y.

J.G. Shute, D.V.M.; Doylestown, Pa.

Dr. Alan G. Shriro; Berkeley Dog & Cat Hospital; Berkeley, Calif.

Albert Singer, V.M.D.; Morris Plains, N.J.

Avery L. Smith, D.V.M.; Hurley Veterinary Hospital; Kingston, N.Y.

Stafford Veterinary Hospital, P.A.; Manahawkin, N.J.

Anton M. Stenzler, D.V.M.; Pearl River Animal Hospital, Pearl River, N.Y.

William Stroh, V.M.D.; Hopewell Veterinary Hospital; Hollywood, Pa.

Dr. J.L. Tait; 145th St. Animal Center, Inc.; New York, N.Y.

David Tayman, D.V.M.; Columbia Animal Hospital; Columbia, Md.

Dr. James W. Tompsett; South Towne Veterinary Hospital; Rochester, N.Y.

Louis Tumolo Jr., D.V.M., P.C.; Rhinebeck Animal Hospital; Rhinebeck, N.Y.

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Dr. Richard Yacowitz; Raritan Hospital for Animals; Edison, N.J.

Dr. Gary Yarnell; Harmon Animal Hospital; Rye, N.Y.

Dr. Frederick M. Zander; Ballard Animal Hospital; Solvang, Calif.

Dr. Howard Zweighaft; Triboro Animal Hospital; Bronx, N.Y.

(Additional memorial cards will be sent upon request.)

HERE'S GOOD NEWS:

Income tax rates are going down in 1982.

...AND MORE GOOD NEWS:

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ELISA (Cont. from page 1)

each test sample can be made and subjectivity inherent in the immunofluorescent procedure is eliminated. All manipulations except for the addition of test samples to the wells are semi-automated, and the total time required to perform the ELISA is under two hours.

A computer-assisted kinetics-based ELISA program in use at the New York State Diagnostic Laboratory has a capability of performing hundreds of immunoassays per day, and an evaluation of our ELISA using this system is currently in progress. A nomograph comparing ELISA slope values with immunofluorescent assay titers is being generated which will be capable of converting any slope value to its equivalent titer on a continuous scale (e.g., a standard titer of 1:400 will be read out more precisely as 1:650, or 1:1600 as 1:1250, etc.). Thus rising titers to coronavirus can be followed over much shorter periods of time and with greater precision than has been possible in the past. Conversion of slope values back to titers will allow continued use of an established scale of measurement so that no new system of values (i.e., ELISA slopes) need be introduced.

In addition to TGEV, we are evaluating the use of cross-reacting canine coronavirus (CCV) as a test antigen in this assay. Preliminary data indicate that good correlation with immunofluorescent assay titers is also obtained with CCV.

[1]Barlough, J.E., R.H. Jacobson, K.L. Marcella, and F.W. Scott: A semi-automated kinetics-based ELISA for feline coronaviral antibodies cross-reactive with transmissible gastroenteritis virus of swine. Manuscript in preparation.

Dr. Jeffrey E. Barlough (Davis '79) is studying for his Ph.D. in Veterinary Virology, with emphasis on the cat's immune response to feline infectious peritonitis (FIP).

Dr. Richard H. Jacobson earned his Ph.D. in Zoology at Montana State University in 1975. He is an Assistant Professor of Immunoparasitology and the head of a developmental serology laboratory at Cornell.

Ken L. Marcella is a third-year student at the N.Y.S. College of Veterinary Medicine at Cornell.

Dr. Fredric W. Scott (Cornell '62; Ph.D., Cornell '68) is a Professor of Veterinary Virology and Director of the Cornell Feline Health Center.

Micturition (Cont. from page 5)

because of self-mutilation and impaired circulation to the tail.

[1]Rosin, A.H., and L. Ross: Diagnosis and pharmacological management of disorders of urinary continence in the dog. The Compendium on Continuing Education for the Practicing Veterinarian, 3:601-608, 1981.

Acknowledgement: The authors express appreciation to Dr. J. Guglielmino of Rochester, N.Y., for her assistance in several of the cases we studied.

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